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ORIGINAL ARTICLE

Association of single nucleotide polymorphism rs7579169 with hypertension disorders during pregnancy and perinatal outcome

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Abstract

BACKGROUND: Hypertension during pregnancy is a heterogeneous group of disorders with elevated blood pressure with or without proteinuria. The multiple researches are held on the subject of a genetic conditioning of preeclampsia and pregnancy induced hypertension.
OBJECTIVES: The study was designed to evaluate the impact of the single nucleotide polymorphism (SNP) rs7579169 on hypertension disorders in pregnancy, especially on PE and PIH as well as on the perinatal outcome.
METHODS: It is a case–control study. The study included 104 women with uncomplicated pregnancies in the control group and 75 pregnant women with hypertension disorders in the study group, hospitalized in the Perinatology and Obstetrics Department of the University Hospital in Cracow. Genomic DNA was extracted from peripheral blood leukocytes and SNP rs7579169 was genotyped from all patients. We analyzed the genotypes distribution and allele frequencies of polymorphism rs7579169 and its association with perinatal outcome in all groups. A p-value <0.05 was considered as significant.
RESULTS: Clinical evaluation included standard anthropometric measures like weight and height for the calculation of the body mass index in the beginning and in the end of the pregnancy, blood pressure, time and a method of delivery, birth weight, Apgar score. The heterozygote CT was associated with a 4.5-fold increased risk of preeclampsia in pregnant patients. The presence of TT genotype significantly increased the risk of intrauterine growth restriction (<10 percentile).
CONCLUSIONS: The study show probable impact of SNP rs7579169 on pregnancy, but further studies on larger groups are needed.

Abbreviations:

BMI	- body mass index
CG	- control group
CH	- chronic hypertension
eNOS	- endothelial nitric oxide synthase
GWAS	- genome-wide association
GWLS	- Genome-wide linkage screens
INHBB	- inhibin beta B
MAP	- mean arterial pressure
NHBPEP-WG	- the National High Blood Pressure Education Program Working Group
PE	- preeclampsia
PIH	- pregnancy induced hypertension
SNP	- single nucleotide polymorphism

INTRODUCTION

Hypertension during pregnancy is a heterogeneous group of disorders with elevated blood pressure with or without proteinuria. It is one of the most common pregnancy complication, responsible for high mortality and morbidity both of a mother and a child (Langenveld *et al* 2011; Aali *et al* 2004). In 10% to 15% preeclampsia and eclampsia are responsible for direct maternal deaths of pregnant women (Duley 2009). There is no other effective treatment than finishing the pregnancy, as the expectant management of severe preeclampsia before 25 weeks of gestation is complicated with nearly 95% intrauterine fetal loss rate (Sezik *et al.* 2007). All over the world multiple researches are held among others on the subject of genetic conditioning of preeclampsia and pregnancy induced hypertension. Although many information has been gathered, still the fully understanding of etiology is unknown. The usage of the identified risk factors of the disease is limited and in the stage of the clinic study, what is connected with the complexity of the disease itself. Many variables, like environment, diet, style of life, socioeconomical conditions and stress have an impact on the genetic expression and the progress of the disease. The model of genetic heredity of preeclampsia, pregnancy induced hypertension or essential hypertension is not ready yet, despite many years' studies (Williams & Morgan 2012).

Genetic polymorphism is an existence of two or more different alleles in one locus in DNA, more often than it is expected, according to mutation frequency in a population. Both the mutation and polymorphism is a qualitative and/or quantitative change in the genetic material. Single nucleotide polymorphism (SNP) is a point mutation, like insertion, deletion or substitution of one of the nucleotides in coding or uncoding DNA sequence. A wide range of methods of finding polymorphisms is now available, like a genome-wide association scan (GWAS) or Genome-wide linkage screens (GWLS). One study reported the results of the GWAS in which, as the first, a risk locus for preeclampsia on chromosome 2q14, near the inhibin beta B (INHBB) gene was identified. In that study researchers had successfully genotyped 648,175 SNPs in 538 preeclampsia cases and 540 normal pregnancy controls with the usage of the Illumina OmniExpress-12 BeadChip (Johnson *et al.* 2012).

The aim of the present study was evaluation of the impact of the novel SNP rs7579169 located on chromosome 2q14.2, on the preeclampsia and pregnancy induced hypertension as well as on the perinatal outcome.

MATERIAL AND METHODS

179 pregnant patients were randomly enrolled in the study from the women hospitalized in the Obstetrics and Perinatology Department of the University Hospital in Cracow. It is a case-controlled study. Hypertension and proteinuria were defined according to NHBPEP-WG (2000). The hypertensive patients were divided into smaller groups depending on the final diagnosis of preeclampsia (PE), pregnancy induced hypertension (PIH) or chronic hypertension (CH) in number of 29, 35 and 11 patients respectively. The hypertension was considered if the systolic/diastolic blood pressure was ≥140/90 mmHg in two separated measurements. If the proteinuria occurred with hypertension after end of 20th week of pregnancy we diagnosed the preeclampsia. The exclusion criteria included: multiple pregnancy, stillbirth, fetal anomalies, patients with renal, liver or heart diseases as well as the withdrawal of patient's consent.

104 normotensive pregnant women were enrolled as a control group (CG) aged 18 to 39 years. Written informed consent was obtained from all the patients enrolled in this study and this study was approved by local Ethic Committee of Jagiellonian University. Each patient was interviewed and detailed medical history like gravidity, parity, week of gestation was taken. Clinical evaluation included standard anthropometric measures like body weight and height for the calculation of the BMI in the beginning and in the end of pregnancy, blood pressure (systolic, diastolic and MAP), time and a method of the delivery, the fetal birth weight, the Apgar score.

Samples of venous blood from all the patients were collected into EDTA tubes while standard medical procedures during hospitalization. The blood samples were kept at –80 °C for some time as all of the samples were analyzed at the same time. Genomic DNA was extracted from peripheral blood leukocytes using the standardized protocols (NHBPEP-WG 2000). The DNA extraction was performed with High Pure PCR Template Preparation Kit. The DNA quality and quantity was assessed on a spectrophotometer NanoDrop ND-1000. DNA was amplified by PCR with primers designed in the Primer 3 Input program (v. 0.4.0): P-F1: 5'-CTGCTCTATGGCTTCCCAAG-3' P-R1: 5'-TGAGCGAACTCAGGCACA-3'. Genotyping was performed by investigators blinded to clinical status.

The characteristics of the study groups were compared properly by Kruskal-Wallis test, Mann-Whitney test, Fisher's exact test, chi-square test, Classical analysis of variance and post-hoc Tuckey test. The distribution of the genotype variants among studied subjects were compared by chi-squared analysis and test Fisher's exact. Odds ratios were calculated as a measure of the association between the genotypes and clinical phenotypes. For each odds ratio, *P* values and 95% confidence intervals were calculated. A P value of <0.05 was considered as statistically significant. The results were analyzed with IBM SPSS Statistics v.20.

RESULTS

The clinical characteristics of the studied population are presented in Table 1. Pregnant women with hypertension and normotensive pregnant patients were

Tab. 1. Summary of patient characteristics.

	CH (n=11)	PIH (n=35)	PE (n=29)	CG (n=104)	*p*-value
Primigravid, n, %	4, 36.4%	16, 45.7%	16, 55.2%	48, 46.2%	p^{chi}=0.7
Nulliparous, n, %	6, 54.5%	20, 57.1%	19, 65.5%	56, 53.8%	p^{F}=0.7
Pregnancy loss, n, %	3, 27.3%	7, 20.0%	6, 20.7%	15, 14.4%	p^{F}=0.5
Height, cm mean±SD min – max	n=10 165.3±6.6 155–174	n=34 165.9±6.1 150–176	n=27 163.1±6.2 150–175	n=85 165.8±6.5 150–186	p^{A}=0.3
Maternal age, years mean±SD min – max	34.9±3.8 28–39	30.6±6.2 21–44	30.5±4.3 25–39	29.1±4.4 18–39	p^{MW}=0.004 (1)
Body weight before pregnancy, kg mean± SD min – max	n=10 83.1±23.0 54–117	n=31 69.2±12.3 48–104	n=25 61.1±13.8 45–93	n=77 62.7±9.8 42–86	p^{KW}=0.001 (2)
BMI before pregnancy, kg/m^2 mean±SD min – max	n=10 30.3±7.8 21.6–41.5	n=31 25.3±5.1 17.9–41.7	n=25 22.8±4.2 17.2–32.2	n=77 22.8±3.4 16.8–31.6	p^{KW}=0.002 (3)
Weight gain during pregnancy, kg mean±SD min – max	n=10 9.7±9.2 –15do18	n=31 15.2±6.2 6–30	n=25 16.0±7.1 6–36	n=78 14.9±5.1 [5–32]	p^{KW}=0.2

A – Classical analysis of variance; BMI – Body mass index; CG – control group; CH – chronic hypertension; chi – chi-square test, df=3; F – Fisher's exact test; KW – Kruskal-Wallis test; MW – Mann-Whitney test; PE – preeclampsia; PIH – pregnancy induced hypertension; SD –standard deviation

(1) post-hoc Tuckey test: chronic hypertension group vs. gestational hypertension p=0.045; chronic hypertension vs. preeclampsia p=0.044; chronic hypertension vs. the control group, p= 0.001; other pairwise comparisons p>0.05

(2) post-hoc Tuckey test chronic hypertension group vs. gestational hypertension p=0.012; chronic hypertension vs. preeclampsia P<0.001; chronic hypertension vs. control group, p<0.001, other pairwise comparisons p>0.05

(3) post-hoc Tuckey test chronic hypertension vs. gestational hypertension p=0.011; chronic hypertension vs preeclampsia p<0.001; chronic hypertension vs. control group p<0.001; gestational hypertension vs. control group p=0.028; other pairwise comparisons p>0.05

Tab. 2. Perinatal outcomes.

	CH	PIH	PE	CG	*p*-value
Gestational age at delivery, weeks mean±SD min - max	n=11 35.0±5.1 26–41	n=35 38.1±27 28–41	n=29 35.1±3.9 26–40	n=104 39.6±1.3 36–42	p^{KW}<0.001 (1)
Infant birth weight, g mean±SD min – max	n=11 2213±999 920–3710	n=35 3067±840 720–4130	n=29 2227±955 590–4400	n=104 3485±413 2500–4430	p^{KW}<0.001 (2)
Intrauterine growth restriction, n, %					
Eutrophic child	6, 54.5%	27, 77.1%	13, 44.8%	91, 88.3%	p^{F}<0.001
Hypotrophic child	5, 45.5%	8, 22.9%	16, 55.2%	12, 11.7%	

CG – control group; CH – chronic hypertension; F – Fisher's exact test; KW – Kruskal-Wallis test; PE – preeclampsia; PIH – pregnancy induced hypertension; SD – standard deviation

(1) post-hoc Tuckey test: chronic hypertension group vs. gestational hypertension, p=0.003; chronic hypertension vs controls, p<0.001; gestational hypertension vs. preeclampsia, p<0.001; gestational hypertension vs controls, p=0.012; preeclampsia vs control, p<0.001 , the remaining pairwise comparisons p>0.05

(2) post-hoc Tuckey test: chronic hypertension group vs. gestational hypertension p=0.002; chronic hypertension vs controls, p<0.001; gestational hypertension vs. preeclampsia p<0.001 vs control preeclampsia p<0.001, other pairwise comparisons p>0.05

homogeneous for gestational history and height, but significant differences were found in the age, blood pressure (systolic, diastolic and mean p<0.001) and weight measurements before and during pregnancy. The gestational age at the birth and birth weight were significantly lower in preeclampsia and chronic hypertension (Table 2).

The rs7579169 polymorphism was in Hardy-Weinberg equilibrium. We analyzed the genotypes distribution and allele frequencies of polymorphism rs7579169 in all groups (the data summarized in Table 3). There were no significant differences in genotype frequencies between the hypertensive groups and the control panel when analyzing data under a codominant (TT, TC, CC)

and dominant (CT+CC versus TT) model. However, in the recessive model (CC frequency versus TT+CT frequency) the difference between the groups was found statistically significant (p=0.018). Moreover, the allele C was most common in patients with chronic hypertension (86.4%) (Table 3).

Different clinical parameters like weight changes during pregnancy were then compared among the three groups defined by the polymorphism genotypes. The results of this comparison, given in Table 4, show a significant relationship between the presence of TT genotype and the weight gain and BMI changes during pregnancy. Also interestingly the presence of TT genotype showed significantly increased risk of IUGR <10 percentile (OR=4.30, 95% CI=1.2–15.1, p=0.023) in the multidimensional model standardized for maternal age, the incidence of miscarriages in the past, mothers' BMI in early pregnancy (Table 5). It was shown in Table 6 that the heterozygote CT was associated with a 4.5-fold increased risk of preeclampsia in the multidimensional model (OR=4.59; 95% CI 1.31–16.1, p=0.01).

No association was found between this polymorphism and the blood pressure (mean, systolic or diastolic), the Apgar score and other analyzed data (data not shown).

DISCUSSION

Hypertensive disease during pregnancy is a crucial perinatal problem. It has been noticed that preeclampsia occurs more often among people who are related, which was the cause of significant suspicion of hereditary predisposition (Cnattingius *et al.* 2004). Preeclampsia has a familial association as it was shown that daughters of PE affected women were having more than twice the risk of developing preeclampsia. What is more, the maternal association is stronger than the fetal association. The familial association predicts more severe pre-eclampsia

Tab. 3. Distribution of the polymorphism rs7579169 genotype and alleles between study groups and normal pregnancy women.

	CH	PIH	PE	CS	*p*-value
Codominant, n, %					
CC	8, 72.7%	11, 31.4%	6, 20.7%	42, 40.4%	p^F=0.1
CT	3, 27.3%	18, 51.4%	17, 58.6%	49, 47.1%	
TT	0, 0.0%	6, 17.1%	6, 20.7%	13, 12.5%	
Recessive, n,%					
CC	8, 72.7%	11, 31.4%	6, 20.7%	42, 40.4%	p^F=0.02
TT+CT	3, 27.3%	24, 68.6%	23, 79.3%	62, 59.6%	
Dominant, n, %					
CT+CC	11, 100.0%	29, 82.9%	23, 79.3%	91, 87.5%	p^F=0.4
TT	0, 0.0%	6, 17.1%	6, 20.7%	13, 12.5%	
Alleles, n, %					
C	19, 86.4%	40, 57.1%	29, 50.0%	133, 63.9%	df=3
T	3, 13.6%	30, 42.9%	29, 50.0%	75, 36.1%	p^{chi}=0.02

CG – control group; CH – chronic hypertension; chi – chi-square test; F – Fisher's exact test; PE – preeclampsia; PIH – pregnancy induced hypertension;

Tab. 4. Weight changes during pregnancy depending on the polymorphism rs7579169 genotype.

	CC (n=56)	CT (n=68)	TT (n=19)	*p*-value
Weight gain during pregnancy, kg mean±SD min – max	14.5±6.5 –15do30	14.2±5.3 6–30	17.9±7.5 9–36	P^A=0.06
Weight gain during pregnancy, % mean±SD min – max	23.1±10.9 –15–54.8	22.7±9.7 6.7–50.0	30.2±12.2 14.3–57.1	P^A=0.02 (1)
BMI change during pregnancy, kg/m^2 mean±SD min – max	5.3±2.3 –5.4–9.9	5.2±1.8 2.1–10.8	6.6±2.9 2.8–14.1	P^A=0.04 (2)

A – Classical analysis of variance; BMI – Body mass index; SD – standard deviation;
(1) post-hoc Tuckey test, group CC vs TT p=0.032; CT vs TT p=0.019;
(2) post-hoc Tuckey test, group CT vs TT p=0.038;

Tab. 5. The risk of the intrauterine growth restriction depending on the polymorphism rs7579169 genotype.

	OR[1]	95% CI		*p*-value	OR[2]	95% CI		*p*-value	OR[3]	95% CI		*p*-value
CC	1				1				1			
CT	1.46	0.7	3.2	0.4	1.9	0.77	4.66	0.2	1.4	0.5	3.8	0.5
TT	2.29	0.8	6.6	0.1	4.3	1.23	15.1	0.02	5.26	1.3	21.1	0.02

CI – confidence interval; OR – odds ratio, logistic regression model, 1 – dimensional model, 2 – model multi-dimensional, standardized for maternal age, the incidence of miscarriages in the past, BMI mothers in early pregnancy; 3 – model multi-dimensional, standardized variables of model 2 and the average values of pressure, the presence of proteinuria

Tab. 6. The risk preeclampsia depending on the polymorphism rs7579169 genotype.

	OR[1]	95% CI		*p*-value	OR[2]	95% CI		*p*-value	OR[3]	95% CI		*p*-value
CC	1				1				1			
CT	2.43	0.88	6.72	0.1	4.59	1.31	16.1	0.017	5.31	1.47	19.2	0.01
TT	3.23	0.89	11.8	0.1	3.57	0.74	17.3	0.1	3.15	0.63	15.7	0.16

CI – confidence interval ; OR – odds ratio, logistic regression model, 1 – dimensional model; 2 – model multidimensional, standardized for maternal age, the incidence of miscarriages in the past, maternal BMI in early pregnancy; 3 – model multidimensional, standardized variables in model 2, diabetes diagnosis (yes / no), hypothyroidism (yes / no), other concomitant diseases (yes / no).

(Skjaerven *et al.* 2005). Among many "genes candidates", which may have an impact on the origin of hypertension disease during pregnancy, include those which products regulate the blood pressure, water-sodium homeostasis and cardiovascular system, like renin-angiotensin-aldosteron system. Currently it is believed, that it is a multigenetic disease with great role of the epigenetic. The pathogenesis of preeclampsia includes abnormalities in the endothelial nitric oxide synthase (eNOS) and the nitric oxide pathway. The Two studies, which have had investigated serum levels of inhibin B in pregnancy, have reported lower (Petraglia *et al.* 1997) or no difference (Yair *et al.* 2001) in maternal serum in preeclamptic women as compared to normotensive pregnancies. Inhibin beta B is a subunit of the inhibin B. The novel polymorphism rs7579169 is located on chromosome 2q14.2, close to the Inhibin beta B (INHBB) gene. Its role is still unknown. Polymorphism rs7579169 was first found in performed genome-wide association study (GWAS) for preeclampsia in unrelated Australian individuals of the Caucasian ancestry (Johnson *et al.* 2012). The researchers were not able to replicate the associations in cohorts from Norway and Finland, concluding that it is more likely for the SNP to be in linkage disequilibrium with unidentified causal variant(s). The authors found no literature about the clinical significance or possible pathomechanism of analyzed SNP. Nevertheless, on the base of the present study some conclusions can be made, especially about mother gain weight and perinatal outcomes.

The polymorphism rs7579169 may play a role in different aspects of pregnancy like the change of the weight gain of the pregnant woman on the one side, as well as on the growth of the fetus and birth weight of the child on the other. The presence of genotype TT was associated with bigger mother weight gain during pregnancy and increased risk of fetal hypotrophy. The significant differences between the genotypes distribution and allele frequencies of polymorphism rs7579169 in the analyzed groups show the probable influence of the studied SNP on the risk of the hypertension during pregnancy. The probable impact is shown especially on the preeclampsia, while heterozygote CT of the SNP rs7579169 was associated with even a 4.5-fold increased risk of the disease taking into account some confounding variables. While these results do not directly support any possible pathomechanism between the novel SNP and preeclapmsia, this study may contribute to better understanding of the subtle interactions between the polymorphic genetic structure and the pregnancy complication risks. More studies on larger groups are needed.

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